Claims

What is claimed is:

1. A method of producing a heterologous biological substance, comprising:

(a) cultivating a mutant of a parent *Aspergillus niger* strain in a medium suitable for the production of the heterologous biological substance, wherein (i) the mutant strain comprises a first nucleotide sequence encoding the heterologous biological substance and one or more second nucleotide sequences comprising a modification of *glaA* and at least one of the genes selected from the group consisting of *asa*, *amyA*, *amyB*, *prtT*, and *oah*, and (ii) the mutant strain is deficient in the production of glucoamylase and at least one enzyme selected from the group consisting of acid stable alpha-amylase, neutral alpha-amylase A, and neutral alpha-amylase B, protease, and oxalic acid hydrolase compared to the parent *Aspergillus niger* strain when cultivated under identical conditions; and

(b) recovering the heterologous biological substance from the cultivation medium.

2. The method of claim 1, wherein at least one of the genes is *asa*.

3. The method of claim 1, wherein at least one of the genes is *amyA*.

4. The method of claim 1, wherein at least one of the genes is *amyB*.

5. The method of claim 1, wherein at least one of the genes is *prtT*.

6. The method of claim 1, wherein at least one of the genes is *oah*.

7. The method of claim 1, wherein the biological substance encoded by the first nucleotide sequence is a biopolymer.

8. The method of claim 7, wherein the biopolymer is selected from the group consisting of a nucleic acid, polyamide, polyamine, polyol, polypeptide, and polysaccharide.

9. The method of claim 8, wherein the polypeptide is selected from the group consisting of an antigen, enzyme, growth factor, hormone, immunodilator, neurotransmitter, receptor, reporter

protein, structural protein, and transcription factor.

10. The method of claim 9, wherein the enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase, or ligase.

11. The method of claim 8, wherein the polysaccharide is chitin, heparin, or hyaluronic acid.

12. The method of claim 1, wherein the biological substance encoded by the first nucleotide sequence is a metabolite.

13. The method of claim 1, wherein the first nucleotide sequence comprises a biosynthetic or metabolic pathway.

14. The method of claim 1, wherein the mutant strain comprises at least two copies of the first nucleotide sequence encoding a biological substance.

15. The method of claim 1, wherein the mutant strain produces at least 25% less glucoamylase and one or more enzymes selected from the group consisting of acid stable alpha-amylase, neutral alpha-amylase A, and neutral alpha-amylase B, protease, and oxalic acid hydrolase compared to the parent *Aspergillus niger* strain when cultivated under identical conditions.

16. The method of claim 1, wherein the mutant strain is completely deficient in glucoamylase and at least one enzyme selected from the group consisting of acid stable alpha-amylase, neutral alpha-amylase A, and neutral alpha-amylase B, protease, and oxalic acid hydrolase compared to the parent *Aspergillus niger* strain when cultivated under identical conditions.

17. The method of claim 1, wherein the mutant strain further comprises a modification of one or more genes which encode a proteolytic activity.

18. The method of claim 17, wherein the proteolytic activity is selected from the group consisting of an aminopeptidase, dipeptidylaminopeptidase, tripeptidylaminopeptidase, carboxypeptidase, aspergillopepsin, serine protease, metalloprotease, cysteine protease, and vacuolar protease.

19. The method of claim 1, wherein the mutant strain further comprises a modification of one or more genes encoding an enzyme selected from the group consisting of a carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, galactosidase, beta-galactosidase, glucose oxidase, glucosidase, haloperoxidase, hemicellulase, invertase, isomerase, laccase, ligase, lipase, lyase, mannosidase, oxidase, pectinolytic enzyme, peroxidase, phytase, phenoloxidase, polyphenoloxidase, ribonuclease, transferase, alpha-1,6-transglucosidase, transglutaminase, and xylanase.

20. A mutant of a parent *Aspergillus niger* strain, comprising a first nucleotide sequence encoding a heterologous biological substance and one or more second nucleotide sequences comprising a modification of *glaA* and at least one of the genes selected from the group consisting of *asa, amyA, amyB*, *prtT* and *oah*, wherein the mutant strain is deficient in glucoamylase and at least one enzyme selected from the group consisting of acid stable alpha-amylase, neutral alpha-amylase A, and neutral alpha-amylase B, protease, and oxalic acid hydrolase compared to the parent *Aspergillus niger* strain when cultivated under identical conditions.

21. The mutant strain of claim 20, wherein at least one of the genes is *asa*.

22. The mutant strain of claim 20, wherein at least one of the genes is *amyA*.

23. The mutant strain of claim 20, wherein at least one of the genes is *amyB*.

24. The mutant strain of claim 20, wherein at least one of the genes is *prtT*.

25. The mutant strain of claim 20, wherein at least one of the genes is *oah*.

26. The mutant strain of claim 20, wherein the biological substance encoded by the first nucleotide sequence is a biopolymer.

27. The mutant strain of claim 26, wherein the biopolymer is selected from the group consisting of a nucleic acid, polyamide, polyamine, polyol, polypeptide, and polysaccharide.

28. The mutant strain of claim 27, wherein the polypeptide is selected from the group consisting of an antigen, enzyme, growth factor, hormone, immunodilator, neurotransmitter, receptor, reporter protein, structural protein, and transcription factor.

29. The mutant strain of claim 28, wherein the enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase, or ligase.

30. The mutant strain of claim 20, wherein the biological substance encoded by the first nucleotide sequence is a metabolite.

31. The mutant strain of claim 20, wherein the first nucleotide sequence comprises a biosynthetic or metabolic pathway.

32. The mutant strain of claim 20, which comprises at least two copies of the first nucleotide sequence encoding a biological substance.

33. The mutant strain of claim 20, which produces at least 25% less glucoamylase and one or more enzymes selected from the group consisting of acid stable alpha-amylase, neutral alpha-amylase A, and neutral alpha-amylase B, protease, and oxalic acid hydrolase compared to the parent *Aspergillus niger* strain when cultured under identical conditions.

34. The mutant strain of claim 20, which is completely deficient in glucoamylase and at least one enzyme selected from the group consisting of acid stable alpha-amylase, neutral alpha-amylase A, and neutral alpha-amylase B, protease, and oxalic acid hydrolase compared to the parent *Aspergillus niger* strain when cultured under identical conditions.

35. The mutant strain of claim 20, which further comprises a modification of one or more genes which encode a proteolytic activity.

36. The mutant strain of claim 35, wherein the proteolytic activity is selected from the group consisting of an aminopeptidase, dipeptidylaminopeptidase, tripeptidylaminopeptidase, carboxypeptidase, aspergillopepsin, serine protease, metalloprotease, cysteine protease, and vacuolar protease.

37. The mutant strain of claim 20, which further comprises a modification of one or more genes encoding an enzyme selected from the group consisting of a carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, galactosidase, beta-galactosidase, glucose oxidase, glucosidase, haloperoxidase, hemicellulase, invertase, isomerase, laccase, ligase, lipase, lyase, mannosidase, oxidase, pectinolytic enzyme, peroxidase, phytase, phenoloxidase, polyphenoloxidase, ribonuclease, transferase, alpha-1,6-transglucosidase, transglutaminase, and xylanase.

38. A method for obtaining a mutant of a parent *Aspergillus niger* strain, comprising:

(a) introducing into the parent *Aspergillus niger* strain a first nucleotide sequence encoding a heterologous biological substance and one or more second nucleotide sequences comprising a modification of *glaA* and at least one of the genes selected from the group consisting of *asa, amyA, amyB, prtT* and *oah*; and

(b) identifying the mutant strain from step (a) comprising the modified nucleotide sequence, wherein the mutant strain is deficient in the production of glucoamylase and at least one enzyme selected from the group consisting of acid stable alpha-amylase, neutral alpha-amylase A, and neutral alpha-amylase B, protease, and oxalic acid hydrolase compared to the parent *Aspergillus niger* strain when cultivated under identical conditions.

39. The method of claim 38, wherein at least one of the genes is *asa*.

40. The method of claim 38, wherein at least one of the genes is *amyA*.

41. The method of claim 38, wherein at least one of the genes is *amyB*.

42. The method of claim 38, wherein at least one gene of the genes is *prtT*.

43. The method of claim 38, wherein at least one of the genes is *oah*.

44. The method of claim 38, wherein the biological substance encoded by the first nucleotide sequence is a biopolymer.

45. The method of claim 44, wherein the biopolymer is selected from the group consisting of a nucleic acid, polyamide, polyamine, polyol, polypeptide, and polysaccharide.

46. The method of claim 45, wherein the polypeptide is selected from the group consisting of an antigen, enzyme, growth factor, hormone, immunodilator, neurotransmitter, receptor, reporter protein, structural protein, and transcription factor.

47. The method of claim 46, wherein the enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase, or ligase.

48. The method of claim 45, wherein the polysaccharide is chitin, heparin, or hyaluronic acid.

49. The method of claim 38, wherein the biological substance encoded by the first nucleotide sequence is a metabolite.

50. The method of claim 38, wherein the first nucleotide sequence comprises a biosynthetic or metabolic pathway.

51. The method of claim 38, wherein the mutant strain comprises at least two copies of the first nucleotide sequence encoding a biological substance.

52. The method of claim 38, wherein the mutant strain produces at least 25% less glucoamylase and one or more enzymes selected from the group consisting of acid stable alpha-amylase, neutral alpha-amylase A, and neutral alpha-amylase B, protease, and oxalic acid hydrolase compared to the parent *Aspergillus niger* strain when cultured under identical conditions.

53. The method of claim 38, wherein the mutant strain is completely deficient in glucoamylase and one or more enzymes selected from the group consisting of acid stable alpha-amylase, neutral alpha-amylase A, and neutral alpha-amylase B, protease, and oxalic acid hydrolase compared to the parent *Aspergillus niger* strain when cultured under identical conditions.

54. The method of claim 38, wherein the mutant strain further comprises a modification of one or more genes which encode a proteolyic activity.

55. The method of claim 54, wherein the proteolytic activity is selected from the group consisting of an aminopeptidase, dipeptidylaminopeptidase, tripeptidylaminopeptidase, carboxypeptidase, aspergillopepsin, serine protease, metalloprotease, cysteine protease, and vacuolar protease.

56. The method of claim 38, wherein the mutant strain further comprises a modification of one or more genes encoding an enzyme selected from the group consisting of a carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, galactosidase, beta-galactosidase, glucose oxidase, glucosidase, haloperoxidase, hemicellulase, invertase, isomerase, laccase, ligase, lipase, lyase, mannosidase, oxidase, pectinolytic enzyme, peroxidase, phytase, phenoloxidase, polyphenoloxidase, ribonuclease, transferase, alpha-1,6-transglucosidase, transglutaminase, and xylanase.